wherein said third color and said fourth color combine to form a visually or electronically distinguishable color different from both said third color and said fourth color.

REMARKS

The Invention

The present invention relates to methods of detecting the presence of a single copy of a target nucleic acid in a sample by detecting an optical characteristic of a first quantum dot and a second quantum dot attached to the single copy of the target nucleic acid. The first quantum dot and the second quantum dot are preferably distinguishable from each other in the experiment used to detect them. In some embodiments, the single copy of the target nucleic acid is detected by resolving the optical characteristic of at least two quantum dots attached to the single copy of the target nucleic acid from an optical characteristic of a quantum dot not attached to the single copy of the target nucleic acid. In some embodiments of the invention, the first quantum dot and the second quantum dot are visually distinguishable as a first color and a second color. In some embodiments of the present invention, the first quantum dot and the second quantum dot combine to form a third color that is distinguishable from the color of the first quantum dot and the color of the second quantum dot.

Status of the Claims

After entry of this amendment, claims 1-31 are pending. Claims 1, 2-6, 8-9, 15-17, and 18 have been amended. Claims 19-31 have been added. The amendments do not introduce new matter or raise new issues that would require further consideration and/or search.

Amended claim 8 recites "the target nucleic acid," thus correcting a grammatical error. As amended, claim 5 recites "wherein the primer is biotinylated". Amended claims 1-4, 6, 9, and 16-18 recite "target nucleic acid".

Amended claims, 1, 17, and 18 recite "a method of detecting the presence of a single copy of a target nucleic acid in a sample". Claims 1 and 17 further recite "detecting an optical characteristic of a first quantum dot and a second quantum dot attached to said single copy of said target nucleic acid". Support for these amendments is found throughout the specification, for example, on page 14, lines 30-31; page 18, lines 12-22; and page 55, lines 21-28. Thus, no new matter is added by these amendments.

Claims 2, 15, and 16 have been amended to ensure correct antecedent basis in view of the amendments to claim 1.

New claims 19-31 have been added. New claims 19-31 find support in the specification and claims as originally filed. In particular, new claims 19-31 find support in the specification at page 18, line 11 to page 22, line 5; page 56, lines 15-23; and page 58, line 30 to page 59, line 2.

A version of the claims with markings to show changes to the claims are provided in Appendix A. All of the pending claims are provided in Appendix B for the Examiner's convenience.

Objection to the Specification

The specification has been objected to for alleged informalities. Specifically, the Examiner objects to the first paragraph of the specification which states that "The present invention is a continuation-in-part of U.S. Patent Application Serial Number 01/05164 filed on February 16, 2001." As suggested by the Examiner, the specification has been amended to state that "The present invention is a continuation-in-part of U.S. Patent Application Serial Number 09/784,866 filed on February 15, 2001." Applicants respectfully request that the Examiner withdraw the objection to the specification.

Rejection Under 35 U.S.C. § 112, Second Paragraph

Claims 1-18 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the

subject matter which applicant regards as the invention. Applicants respond with amendment and traverse. As set forth in MPEP § 2173.02, "[d]efiniteness of claim language, must be analyzed in light of (A) content of the application; (B) the teachings of the prior art; and (C) the claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made."

In the instant case, the specification adequately defines the terms or the terms are adequately understood by one of skill in the art, such that the claims are not indefinite under 35 U.S.C. §112, second paragraph. Several bases of indefiniteness were raised, and they will be discussed in turn.

1. Claims 1-16

Claims 1-16 are allegedly indefinite for the recitation in claim 1 of "wherein the detection of fluorescence in the sample indicates the presence of at least one target nucleic acid". Claim 1 has been amended as suggested by the Examiner to recite "detecting". Accordingly, Applicants respectfully request that the rejection be withdrawn.

2. Claim 5

Claim 5 is allegedly indefinite for the recitation "the primer comprises a biotinylated primer". Claim 5 has been amended as suggested by the Examiner to recite "wherein the primer is biotinylated". Accordingly, Applicants respectfully request that this aspect of the rejection be withdrawn.

3. Claims 15 and 16

Claims 15 and 16 are allegedly indefinite for the recitation "scanning the substrate with a resolution capable of detecting fluorescence emitted by a single quantum dot". The Examiner alleges that it is unclear whether the recitation refers to detection of a single quantum dot.

As explained above, the present invention relates to methods of detecting the presence of a single copy of a target nucleic acid in a sample. The claims specifically recite that the single copy of a target nucleic acid is detected "by detecting an optical characteristic of a first quantum dot and a second quantum dot attached to the single copy

of the target nucleic acid." Thus, based on the language of the claim, it is clear to one of skill in the art that the recitation refers to detecting an optical characteristic of a first quantum dot and a second quantum dot. Accordingly, Applicants respectfully request that this aspect of the rejection be withdrawn.

4. Claims 17 and 18

Claims 17 and 18 are allegedly indefinite for the recitation "wherein the detection of fluorescence in the sample indicates the presence of at least one target nucleic acid". Claims 17 and 18 have been amended as suggested by the Examiner to recite "detecting." Accordingly, Applicants respectfully request that the rejection be withdrawn.

Rejection Under 35 U.S.C. § 102(b)

Claim 1 stands rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Weiss *et al.* (U.S. Patent 5,990,479). In making this rejection, the Examiner alleges that Weiss *et al.* disclose a method of detecting the presence of at least one target nucleic acid in a sample. Applicants respectfully traverse this rejection.

For a rejection of claims under § 102(b) or § 102(e) to be properly founded, the Examiner must establish that a single prior art reference discloses each and every element of the claimed invention. *See, e.g., Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 U.S.P.Q. 81 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987). In *Scripps Clinic & Research Found. v. Genetech, Inc.*, 18 U.S.P.Q.2d 1001 (Fed. Cir. 1991), the Federal Circuit held that "anticipation requires that all of the elements and limitations of the claim are found with a single prior art reference. . . .***There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention***." *Id.* at 1010 (emphasis added). Anticipation can be found, therefore, only when a cited reference discloses ***all*** of the elements, features or limitations of the presently claimed invention.

The present invention relates to methods of detecting the presence of a single copy of a target nucleic acid in a sample by detecting an optical characteristic

emitted by a first quantum dot and a second quantum dot attached to the single copy of the target nucleic acid, thus detecting the single copy of the target nucleic acid.

Weiss *et al.* describe a method of detecting targets labeled with quantum dots. Weiss *et al.* do ***not*** disclose the presently claimed method of counting a *single copy* of a target nucleic acid by detecting an optical characteristic of a first quantum dot and a second quantum dot attached to a single copy of a target nucleic acid from, thereby detecting the single copy of the target nucleic acid.

Weiss *et al.* describe a detection method in which fluorescence emitted from quantum dots attached to a multiple target nucleic acid species is detected by previously known methods; namely, emission from a ***population*** of species is detected to determine the presence of a target species in the sample. The signal detected is the ***average*** of all emissions arising from the collective members of the population ("ensemble detection").

In contrast to the ensemble detection method of Weiss *et al.*, the claims of the present application now expressly recite a method of detecting a single copy of a target nucleic acid by detecting an optical characteristic of a first quantum dot and a second quantum dot attached to the single copy of a target nucleic acid. Weiss *et al.* neither disclose nor suggest detecting an optical characteristic of a first quantum dot and a second quantum dot attached to a single copy of a target nucleic acid.

To understand the contrast between the detection methods of Weiss *et al.* and the counting methods of the present invention, it is useful to envision a series of microarray spots with decreasing concentrations of bound target. In an ensemble detection system, as described in Weiss *et al.*, bound target concentration is proportional to ***average emission intensity***. Accordingly, a target species is detected by measuring total emission intensity and then resolving total emission intensity by determining the density of label across the surface area of the spot.

In contrast to detection of average emission intensity of a plurality of species, the present invention detects individual target nucleic acids one at a time. The specification and figures explicitly state and illustrate that an optical characteristic of a

first quantum dot and a second quantum dot attached to a single copy of a target nucleic acid is detected, thereby detecting the single copy (*see, e.g.*, Figures 6-7, and 17; pages 15-17; page 18, line 11 to page 22, line 5; and page 58, line 30 to page 59, line 2).

As clarified above, Weiss *et al.* do not disclose Applicants' claimed single copy detection methodology and, therefore, cannot fairly be said to disclose every element of the claimed invention. Accordingly, Applicants respectfully request that the rejection be withdrawn.

Rejections Under 35 U.S.C. § 102(e)

Claims 1, 2, and 17 stand rejected under 35 U.S.C. § 102(e) as allegedly anticipated by Castro *et al.* (U. S. Patent No. 6,114,038). In making this rejection, the Examiner alleges that Castro *et al.* disclose a method of detecting the presence of at least one target nucleic acid in a sample.

Claims 1-3, and 17 stand rejected under 35 U.S.C. § 102(e) as allegedly anticipated by Weiss *et al.* (U. S. Patent No. 6,207,392 B1). In making this rejection, the Examiner alleges that Weiss *et al.* disclose a method of detecting the presence of at least one target nucleic acid in a sample. Applicants respectfully traverse these rejections.

As explained above, for a rejection of claims under § 102(e) to be properly founded, the Examiner must establish that a single prior art reference discloses each and every element of the claimed invention.

The present invention relates to methods of detecting the presence of a single copy of a target nucleic acid in a sample by detecting an optical characteristic of a first quantum dot and a second quantum attached to the single copy of the target nucleic acid, thus detecting the single copy of the target nucleic acid.

Castro *et al.* and Weiss *et al.* each disclose a method of detecting targets labeled with quantum dots. Neither Castro *et al.* nor Weiss *et al.* disclose the presently claimed method of counting a ***single copy*** of a target nucleic acid labeled with a quantum dot by detecting an optical characteristic of a first quantum dot and a second quantum dot

attached to a single copy of a target nucleic acid, thereby detecting the single copy of the target nucleic acid.

Castro *et al.* and Weiss *et al.* each describe a detection method in which fluorescence emitted from quantum dots attached to a multiple target nucleic acid species is detected by previously known methods; namely, emission from a ***population*** of species is detected to determine the presence of target species in the sample. The signal detected is the ***average*** of all emissions arising from the collective members of the population ("ensemble detection").

In contrast to the ensemble detection methods of Castro *et al.* and Weiss *et al.*, the claims of the present application now expressly recite a method of detecting a single copy of a target nucleic acid by detecting an optical characteristic of a first quantum dot and a second quantum dot attached to a single copy of a target nucleic acid. Neither Castro *et al.* nor Weiss *et al.* disclose nor suggest detecting an optical characteristic of a first quantum dot and a second quantum dot attached to a single copy of a target nucleic acid.

Castro *et al.* and Weiss *et al.* each describe a method in which the ***average*** emission of a ***plurality*** of species is detected. In contrast to detection of average emission intensity of a plurality of species, the present invention detects individual target nucleic acids one at a time. The specification and figures explicitly state and illustrate that an optical characteristic of a first quantum dot and a second quantum dot attached to a single copy of a target nucleic acid is detected, thereby detecting the single copy (*see, e.g.*, Figures 6-7, and 17; pages 15-17; page 18, line 11 to page 22, line 5; and page 58, line 30 to page 59, line 2).

As clarified above, neither Castro *et al.* nor Weiss *et al.* disclose Applicants' claimed single copy detection methodology and, therefore, cannot fairly be said to disclose every element of the claimed invention. Therefore, Applicants respectfully request that the rejections under 35 U.S.C. § 102(e) be withdrawn.

Rejections Under 35 U.S.C. § 103(a)

The claims have been rejected, in various combinations, under 35 U.S.C. § 103(a) over a number of different references. Applicants respectfully traverse each of the § 103 obviousness rejections. As set forth in M.P.E.P. § 2143, "[t]o establish a *prima facie* case of obviousness, three basic criteria must be met. *First*, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. *Second*, there must be a reasonable expectation of success. *Finally*, the prior art reference (or references when combined) must teach or suggest all the claim elements. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)."

All three elements set forth above must be present in order to establish a *prima facie* case of obviousness. As explained herein below in connection with each of the § 103(a) obviousness rejections, Applicants assert that a *prima facie* case of obviousness has not been established for at least the following reason: the cited art references do not teach or suggest all the claim limitations. Moreover, one of skill in the art would not have any reasonable expectation of success in arriving at the presently claimed method of detecting a single copy of a target nucleic acid by modifying the cited references.

1. Rejection of claims 4-14 over Weiss *et al.* and Söderlund *et al.* in view of Chan *et al.*

Claims 4-14 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Weiss *et al.* (U.S. Patent No. 6,207,392) and Söderlund *et al.* (U.S. Patent No. 6,013,431) in view of Chan *et al.* (*Science*, 281:2016-2018 (1998)). In making this rejection, the Examiner alleges that Weiss *et al.* disclose a method of detecting the presence of at least one target nucleic acid in a sample, but acknowledges that Weiss *et al.* does not specifically teach that the transcribing provides a polymorphic region of DNA. The

Examiner further alleges that Söderlund *et al.* teach that PCR transcription of a target nucleic acid to produce polymorphic regions of DNA and that methods for detecting polymorphic regions are clinically important.

The Combination of References Fails to Teach or Suggest Each Element of the Applicant's Claimed Invention

As discussed in detail above, Weiss *et al.* disclose a method of detecting a population of targets labeled with quantum dots. Weiss *et al.* do ***not*** disclose the presently claimed method of counting a *single copy* of a target nucleic acid by detecting an optical characteristic of a first quantum dot and a second quantum dot attached to a single copy of a target nucleic acid. Söderlund *et al.* and Chan *et al.* do not remedy the defect in Weiss *et al.*

Söderlund *et al.* describe detection of nucleotide variations in a target nucleic acid by ***ensemble*** counting of a ***population*** of amplified, labeled oligonucleotides complementary to the target nucleotide sequence. Söderlund *et al.* does not teach or suggest the presently claimed method of detecting the presence of a ***single copy*** of a target nucleic acid in a sample.

Chan *et al.* describe detection of biological molecules by detection of quantum dots attached to the molecules. In contrast to the presently claimed invention, Chan *et al.* does not teach or suggest detecting a single copy of a target nucleic acid by detecting an optical characteristic of a first quantum dot and a second quantum dot attached to the single copy of the target nucleic acid.

Thus, even if the teachings of Weiss *et al.* were combined with the teachings of Söderlund *et al.* and Chan *et al.*, it would not lead to the claimed invention because none of the references alone or in combination disclose or suggest a method of counting a ***single copy*** of a target nucleic acid by detecting an optical characteristic of a first quantum dot and a second quantum dot attached to a single copy of a target nucleic acid.

One Of Skill In The Art Would Have No Reasonable Expectation of Success in Detecting a Single Copy of a Target Nucleic Acid by Modifying the Cited References

One of skill in the art would have no reasonable expectation of success in modifying the disclosures of the references to arrive at the claimed method of detecting a single copy of a target nucleic acid by detecting an optical characteristic of a first quantum dot and a second quantum dot attached to the single copy of the nucleic acid. There is no disclosure or suggestion in the cited art that two quantum dots attached to a single copy of a target nucleic acid could be detected. Moreover, the cited art provides no guidance regarding detecting an optical characteristic of a first quantum dot and a second quantum dot attached to a single copy of a target nucleic acid. Without the explicit guidance in the specification of the present application regarding detection of optical characteristics of a first quantum dot and a second quantum dot attached to a single copy of a target nucleic acid, one of skill in the art would not have expected that modifying the cited references would successfully arrive at the claimed method of detecting a single copy of a target nucleic acid.

In view of the foregoing remarks, Applicants respectfully submit that the present invention is non-obvious and patentable over Weiss *et al.* (U.S. Patent No. 6,207,392) and Söderlund *et al.* in view of Chan *et al.* Accordingly, Applicants urge the Examiner to withdraw this rejection under 35 U.S.C. § 103(a).

2. Rejection of claims 15 and 16 over Weiss *et al.* and Söderlund *et al.* in view of Chan *et al.* and further in view of Bawendi *et al.*

Claims 15 and 16 are rejected under 35 U.S.C. § 103(a) as unpatentable over Weiss *et al.* (U.S. Patent No. 6,207,392) and Söderlund *et al.* in view of Chan *et al.* and further in view of Bawendi *et al.* (U.S. Patent No. 6,306,610). In making the rejection, the Examiner alleges that Weiss *et al.* teach a method of detecting the presence of at least one target nucleic acid in a sample, but acknowledges that Weiss *et al.* does not teach providing a polymorphic region of DNA by transcribing a target nucleic acid. In

making the rejection, the Examiner further alleges that Söderlund *et al.* teach the clinical importance of detecting genetic polymorphisms, that Chan *et al.* teach that quantum dot labeling solves the problems associated with radioactive labels because the quantum dots are extremely sensitive and able to bind DNA, and that Bawendi *et al.* teach that single quantum dot complexes are detectable and countable. Based on these allegations, the Examiner concludes that the combination of Weiss *et al.*, Söderlund *et al.*, Chan *et al.*, and Bawendi *et al.* renders the claimed invention obvious. Applicants respectfully traverse this rejection.

The Combination of References Fails to Disclose Each Element of the Applicant's Claimed Invention

As discussed above, Weiss *et al.* (U.S. Patent No. 6,207,392), Söderlund *et al.*, and Chan *et al.*, fail to disclose all of the elements of the claimed invention. Bawendi *et al.* do not cure the deficiency of Weiss *et al.* (U.S. Patent No. 6,207,392), Söderlund *et al.*, and Chan *et al.* Bawendi *et al.* describe a detection method in which fluorescence emitted from quantum dots attached to a multiple target nucleic acid species is detected by previously known methods. According to the method of Bawendi *et al.*, emission from a *population* of species is detected to determine the presence of target species in the sample. Bawendi *et al.* contains no teaching or suggestion of detecting the presence of a single copy of a target nucleic acid in a sample by detecting an optical characteristic of a first quantum dot and a second quantum dot attached to the single copy of the target nucleic acid. Therefore, one of skill in the art would not have had the motivation to combine Bawendi *et al.* with Weiss *et al.* (U.S. Patent No. 6,207,392), Söderlund *et al.*, and Chan *et al.*

Even if one of skill in the art were to combine Weiss *et al.* (U.S. Patent No. 6,207,392), Söderlund *et al.*, Chan *et al.*, and Bawendi *et al.* the combination would not lead to the claimed invention because none of the references alone, or in combination teach or suggest the claimed method of detecting the presence of a single copy of a target nucleic acid in a sample by detecting an optical characteristic of a first quantum dot and a second quantum dot attached to a single copy of a target nucleic acid.

One Of Skill In The Art Would Have No Reasonable Expectation of Success in Detecting a Single Copy of a Target Nucleic Acid by Modifying the Cited References

One of skill in the art would have no reasonable expectation of success in modifying the disclosures of the references to arrive at the claimed method of detecting a single copy of a target nucleic acid by detecting an optical characteristic of a first quantum dot and a second quantum dot attached to the single copy of the nucleic acid. There is no teaching or suggestion in the cited art that two quantum dots attached to a single copy of a target nucleic acid could be detected. Moreover, the cited art provides no guidance regarding detecting an optical characteristic of a first quantum dot and a second quantum dot attached to a single copy of a target nucleic acid. Without the explicit guidance in the specification of the present application regarding detection of optical characteristics of a first quantum dot and a second quantum dot attached to a single copy of a target nucleic acid, one of skill in the art would not have expected that modifying the cited references would successfully arrive at the claimed method of detecting a single copy of a target nucleic acid.

In view of the foregoing remarks, Applicants respectfully submit that the present invention is non-obvious and patentable over Weiss *et al.* (U.S. Patent No. 6,207,392) and Söderlund *et al.* in view of Chan *et al.* and further in view of Bawendi *et al.* Accordingly, Applicants urge the Examiner to withdraw this rejection under 35 U.S.C. § 103(a).

Rejection For Obviousness-Type Double Patenting

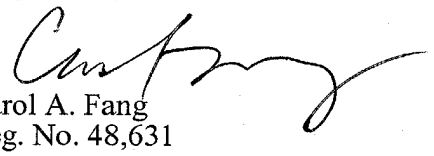
Claims 1-3 and 17 stand rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1, 3, 6, 10, and 11 of U.S. Patent No. 6,274,323 B1 (Bruchez *et al.*). Since the application and the cited patent are commonly owned, Applicants will submit a terminal disclaimer to overcome this rejection after the Examiner determines that the pending claims are otherwise allowable.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (415) 576-0200.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Please insert the following replacement paragraph starting at page 1, line 5.

IN THE SPECIFICATION:

The application is a continuation-in-part of U.S. Patent Application Serial Number [01/05164 filed on February 16, 2001] 09/784,866 filed on February 15, 2001 which claims priority to U.S. Provisional Patent Application Serial Number 60/182,844 filed on February 16, 2000. The application also claims priority to U.S. Provisional Patent Application Serial Number 60/211,054 filed on June 13, 2000. [The] These disclosures [all of which] are incorporated herein in their entirety for all purposes.

IN THE CLAIMS:

1. (Once amended) A method of detecting the presence of [at least one] a single copy of a target nucleic acid [sequence] in a sample, said method comprising:

[labeling at least one target nucleic acid sequence with at least one quantum dot; and

detecting the labeled target nucleic acid sequence by] detecting [fluorescence emitted by the at least one] an optical characteristic of a first quantum dot and a second quantum dot attached to said single copy of said target nucleic acid, wherein said first quantum dot and said second quantum dot are distinguishable, thereby detecting said single copy of said target nucleic acid [wherein the detection of fluorescence in the sample indicates the presence of at least one target nucleic acid].

2. (Once amended) The method as in claim 1, further comprising quantitating the target nucleic acid by analyzing the detected optical characteristic [emitted fluorescence].

3. (Once amended) The method as in claim 1, further comprising transcribing the target nucleic acid [sequence].

4. (Once amended) The method as in claim 3, wherein the target nucleic acid [sequence] comprises DNA and transcribing comprises using a primer which anneals to a conserved region of the DNA and transcribes a polymorphic region of the DNA when extended.

5. (Once amended) The method as in claim 4, wherein the primer [comprises a] is biotinylated [primer] and the transcribing step produces biotinylated DNA.

6. (Once amended) The method as in claim 3, further comprising binding the transcribed target nucleic acid [sequence] to a substrate.

8. (Once amended) The method as in claim 6, further comprising removing unbound portions of the target nucleic acid.

9. (Once amended) The method as in claim 6, further comprising probing the bound target nucleic acid [sequence] using a sequence-tagged hybridization probe.

15. (Once amended) The method as in claim 13, wherein detecting comprises scanning the substrate with resolution capable of detecting [fluorescence emitted by] an optical characteristic of a single quantum [dots] dot.

16. (Once amended) The method as in claim 15, further comprising quantitating the target nucleic acid [sequence] by analyzing the detected [emitted fluorescence] optical characteristic, wherein analyzing comprises counting the number of quantum dots within an area of scanned substrate.

17. (Once amended) A method of detecting the presence of [at least one] a single copy of a target nucleic acid [sequence] in a sample, said method comprising:

[labeling at least one target nucleic acid sequence with at least one quantum dot;

detecting the labeled target nucleic acid by] detecting [fluorescence emitted by the at least one] an optical characteristic of a first quantum dot and a second quantum dot attached to said single copy of said target nucleic acid, wherein said first quantum dot and said second quantum dot are distinguishable, thereby detecting said single copy of said target nucleic acid;

[wherein the detection of fluorescence in the sample indicates the presence of at least one target nucleic acid sequence]

quantitating the target nucleic acid [sequence] by analyzing the detected [emitted fluorescence] optical characteristic.

18. (Once amended) A method of detecting the presence of a single copy of a target nucleic acid in a sample, said method comprising:

transcribing said single copy of said target nucleic acid [sequence] using a primer [that is complementary to a portion of said target nucleic acid sequence and that comprises] comprising an immobilizable label to form an immobilizable target nucleic acid [sequence];

immobilizing said immobilizable target nucleic acid [sequence]on a solid support to form an immobilized target nucleic acid [sequence];

[probing] contacting said immobilized target nucleic acid [using] with a sequence-tagged hybridization probe[, wherein said sequence-tagged hybridization probe is] comprising a nucleic acid complementary to a portion of said target nucleic acid;

[labeling said immobilized target sequence with a quantum dot conjugate, wherein said quantum dot conjugate comprises a quantum dot and a nucleic acid sequence that is complementary to a portion of said sequence-tagged hybridization probe; and

detecting the labeled immobilized target nucleic acid by] detecting [fluorescence emitted by said] an optical characteristic of a quantum dot conjugate comprising a first quantum dot, a second quantum dot, and a nucleic acid sequence complementary to a portion of said sequence-tagged hybridization probe, wherein said first quantum dot and said second quantum dot are distinguishable, thereby detecting said single copy of said target nucleic acid[wherein the detection of fluorescence in said sample indicates said target nucleic acid].

19. (New) The method as in claim 1, wherein said optical characteristic is detected by coincidence detection.

20. (New) The method as in claim 1, further comprising resolving said optical characteristic of said first quantum dot and said second quantum dot attached to said single copy of said target nucleic acid from an optical characteristic of a quantum dot not attached to said single copy of said target nucleic acid.

21. (New) The method as in claim 17, further comprising resolving said optical characteristic of said first quantum dot and said second quantum dot attached to said single copy of said target nucleic acid from an optical characteristic of a quantum dot not attached to said single copy of said target nucleic acid.

22. (New) The method as in claim 18, further comprising resolving said optical characteristic of said quantum dot conjugate from an optical characteristic of a quantum dot conjugate not attached to said immobilized target nucleic acid.

23. (New) The method as in claim 1, wherein said first quantum dot and said second quantum dot are distinguishable by an optical characteristic which is a member selected from the group consisting of fluorescence spectrum, fluorescence emission, fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance, fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof.

24. (New) The method as in claim 1, wherein said optical characteristic is fluorescence.

25. (New) The method as in claim 1, wherein said first quantum dot and said second quantum dot are visually distinguishable as a first color and a second color, respectively.

26. (New) The method as in claim 25, wherein said first color and said second color combine to form a third color that is visually or electronically distinguishable from both said first color and said second color.

27. (New) A method of selecting a mutant DNA away from a wild type DNA, said method comprising:

contacting mutant DNA attached to a first and a second sequence-tagged hybridization probe with a first and a second oligonucleotide tag comprising a sequence complementary to said first and second sequence-tagged hybridization probes and conjugated to a first quantum dot and a second quantum dot, wherein said first quantum dot and said second quantum dot are distinguishable;

contacting wild type DNA attached to a third and a fourth sequence-tagged hybridization probe with a third and fourth oligonucleotide tag comprising a sequence complementary to said third and fourth sequence-tagged hybridization probes and conjugated to a third quantum and a fourth quantum dot, wherein said third quantum dot and said fourth quantum dot are distinguishable; and

detecting an optical characteristic of the quantum dots, whereby detection of said optical characteristic of said first quantum dot and said second quantum dot detects the mutant DNA and detection of said optical characteristic of said third quantum dot and said fourth quantum dot detects wild type DNA.

28. (New) The method as in claim 27, wherein said first quantum dot and said second quantum dot are distinguishable by an optical characteristic which is a member selected from the group consisting of fluorescence spectrum, fluorescence emission, fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance, fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof, and

wherein said third quantum dot and said fourth quantum dot are distinguishable by an optical characteristic which is a member selected from the group consisting of fluorescence spectrum, fluorescence emission, fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance, fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof.

29. (New) The method as in claim 27, wherein said first quantum dot and said second quantum dot are distinguishable by an optical characteristic which is fluorescence; and

wherein said third quantum dot and said fourth quantum dot are distinguishable by an optical characteristic which is fluorescence.

30. (New) The method according to claim 27, wherein said first quantum dot, said second quantum dot, said third quantum dot, and said fourth quantum dot are visually or electronically distinguishable as a first color, a second color, a third color, and a fourth color respectively.

31. (New) The method according to claim 30, wherein said first color and said second color combine to form a visually or electronically distinguishable color different from both said first color and said second color, and

wherein said third color and said fourth color combine to form a visually or electronically distinguishable color different from both said third color and said fourth color.

APPENDIX B
PENDING CLAIMS

1. (Once amended) A method of detecting the presence of a single copy of a target nucleic acid in a sample, said method comprising:

detecting an optical characteristic of a first quantum dot and a second quantum dot attached to said single copy of said target nucleic acid, wherein said first quantum dot and said second quantum dot are distinguishable, thereby detecting said single copy of said target nucleic acid.

2. (Once amended) The method as in claim 1, further comprising quantitating the target nucleic acid by analyzing the detected optical characteristic.

3. The method as in claim 1, further comprising transcribing the target nucleic acid.

4. (Once amended) The method as in claim 3, wherein the target nucleic acid comprises DNA and transcribing comprises using a primer which anneals to a conserved region of the DNA and transcribes a polymorphic region of the DNA when extended.

5. (Once amended) The method as in claim 4, wherein the primer is biotinylated and the transcribing step produces biotinylated DNA.

6. (Once amended) The method as in claim 3, further comprising binding the transcribed target nucleic acid to a substrate.

7. The method as in claim 6, wherein the substrate comprises a streptavidin coated surface, support, plate or slide.

8. (Once amended) The method as in claim 6, further comprising removing unbound portions of the target nucleic acid.

9. (Once amended) The method as in claim 6, further comprising probing the bound target nucleic acid using a sequence-tagged hybridization probe.

10. The method as in claim 9, wherein the target comprises DNA having at least one point mutation and the probing comprises binding the probe to said at least one point mutation of the DNA.

11. The method as in claim 9, wherein the target comprises wild type DNA and the probing comprises binding the probe to the wild type DNA.

12. The method as in claim 9, further comprising removing non-specifically bound probe.

13. The method as in claim 9, wherein each quantum dot has an attached oligonucleotide tag and labeling comprises binding each tag with a complementary sequence of each sequence-tagged hybridization probe.

14. The method as in claim 13, further comprising removing unbound quantum dots.

15. (Once amended) The method as in claim 13, wherein detecting comprises scanning the substrate with resolution capable of detecting an optical characteristic of a single quantum dot.

16. (Once amended) The method as in claim 15, further comprising quantitating the target nucleic acid by analyzing the detected optical characteristic, wherein analyzing comprises counting the number of quantum dots within an area of scanned substrate.

17. (Once amended) A method of detecting the presence of a single copy of a target nucleic acid in a sample, said method comprising:

detecting an optical characteristic of a first quantum dot and a second quantum dot attached to said single copy of said target nucleic acid, wherein said first quantum dot and said second quantum dot are distinguishable, thereby detecting said single copy of said target nucleic acid; and

quantitating the target nucleic acid by analyzing the detected emitted fluorescence.

18. (Once amended) A method of detecting the presence of a single copy of a target nucleic acid in a sample, said method comprising:

transcribing said single copy of said target nucleic acid using a primer comprising an immobilizable label to form an immobilizable target nucleic acid;

immobilizing said immobilizable target nucleic acid on a solid support to form an immobilized target nucleic acid;

contacting said immobilized target nucleic acid [using] with a sequence-tagged hybridization probe comprising a sequence complementary to a portion of said target nucleic acid;

detecting an optical characteristic of a quantum dot conjugate comprising a first quantum dot, a second quantum dot, and a nucleic acid sequence complementary to a portion of said sequence-tagged hybridization probe, wherein said first quantum dot and said second quantum dot are distinguishable, thereby detecting said single copy of said target nucleic acid.

19. (New) The method as in claim 1, wherein said optical characteristic is detected by coincidence detection.

20. (New) The method as in claim 1, further comprising resolving said optical characteristic of said first quantum dot and said second quantum dot attached to said single copy of said target nucleic acid from an optical characteristic of a quantum dot not attached to said single copy of said target nucleic acid.

21. (New) The method as in claim 17, further comprising resolving said optical characteristic of said first quantum dot and said second quantum dot attached to said single copy of said target nucleic acid from an optical characteristic of a quantum dot not attached to said single copy of said target nucleic acid.

22. (New) The method as in claim 18, further comprising resolving said optical characteristic of said quantum dot conjugate from an optical characteristic of a quantum dot conjugate not attached to said immobilized target nucleic acid.

23. (New) The method as in claim 1, wherein said first quantum dot and said second quantum dot are distinguishable by an optical characteristic which is a member selected from the group consisting of fluorescence spectrum, fluorescence emission, fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance, fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof.

24. (New) The method as in claim 1, wherein said optical characteristic is fluorescence.

25. (New) The method as in claim 1, wherein said first quantum dot and said second quantum dot are visually distinguishable as a first color and a second color, respectively.

26. (New) The method as in claim 25, wherein said first color and said second color combine to form a third color that is visually or electronically distinguishable from both said first color and said second color.

27. (New) A method of selecting a mutant DNA away from a wild type DNA, said method comprising:

contacting mutant DNA attached to a first and a second sequence-tagged hybridization probe with a first and a second oligonucleotide tag comprising a sequence complementary to said first and second sequence-tagged hybridization probes and conjugated to a first quantum dot and a second quantum dot, wherein said first quantum dot and said second quantum dot are distinguishable;

contacting wild type DNA attached to a third and a fourth sequence-tagged hybridization probe with a third and fourth oligonucleotide tag comprising a sequence complementary to said third and fourth sequence-tagged hybridization probes and conjugated to a third quantum and a fourth quantum dot, wherein said third quantum dot and said fourth quantum dot are distinguishable; and

detecting an optical characteristic of the quantum dots, whereby detection of said optical characteristic of said first quantum dot and said second quantum dot detects the mutant DNA and detection of said optical characteristic of said third quantum dot and said fourth quantum dot detects wild type DNA.

28. (New) The method as in claim 27, wherein said first quantum dot and said second quantum dot are distinguishable by an optical characteristic which is a member selected from the group consisting of fluorescence spectrum, fluorescence emission, fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance, fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof, and

wherein said third quantum dot and said fourth quantum dot are distinguishable by an optical characteristic which is a member selected from the group consisting of fluorescence spectrum, fluorescence emission, fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance, fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof.

29. (New) The method as in claim 27, wherein said first quantum dot and said second quantum dot are distinguishable by an optical characteristic which is fluorescence; and

wherein said third quantum dot and said fourth quantum dot are distinguishable by an optical characteristic which is fluorescence.

30. (New) The method according to claim 27, wherein said first quantum dot, said second quantum dot, said third quantum dot, and said fourth quantum dot are visually or electronically distinguishable as a first color, a second color, a third color, and a fourth color respectively.

31. (New) The method according to claim 30, wherein said first color and said second color combine to form a visually or electronically distinguishable color different from both said first color and said second color, and

wherein said third color and said fourth color combine to form a visually or electronically distinguishable color different from both said third color and said fourth color.